Effect of repeated pollination upon filled seed in white spruce

RONG H. HO

Ontario Tree Improvement and Forest Biomass Institute, Ontario Ministry of Natural Resources, Maple, Ont., Canada L0J 1E0

Received April 11, 1985[1]
Accepted July 4, 1985

HO, R. H. 1985. Effect of repeated pollination upon filled seed in white spruce. Can. J. For. Res. **15**: 1195–1197.

Repeated pollinations with brush had very little effect on the production of filled seed and pollen from the first pollination was most likely to accomplish fertilization in white spruce (*Picea glauca* (Moench) Voss). Based on the filled seed and the number of coloured pollen on the nucellus, it was suggested that pollinated ovules would stop exuding any pollination drops.

HO, R. H. 1985. Effect of repeated pollination upon filled seed in white spruce. Can. J. For. Res. **15**: 1195–1197.

Des pollinisations répétées à l'aide d'un pinceau eurent peu d'influence dans la production de graines pleines; de plus, le pollen utilisé lors de la toute première pollinisation fut vraisemblablement celui qui provoqua la fécondation pour l'épinette blanche (*Picea glauca* (Moench) Voss). En se basant sur les graines pleines et le nombre de grains de pollen colorés sur le nucelle, l'auteur suggère que les ovules pollinisées arrêteraient toute exudation spécifique des gouttelettes de pollinisation.

[Traduit par le journal]

Introduction

In controlled pollination, seed cones in an isolation bag are often pollinated more than once to increase the number of filled seeds per cone and to pollinate those seed cones which were not pollinated in the previous application. However, the economics of additional pollinations have been questioned since Franklin (1974) reported that in slash pine (*Pinus elliottii*), fertilization was most likely accomplished by the first pollen reaching the seed cones. Similarly, Owens and Simpson (1982) concluded that engulfment of pollen in Douglas-fir (*Pseudotsuga menziesii*) was on a "first-come, first-served" basis.

The first-come, first-served hypothesis is applicable to both Douglas-fir and pines, although they have different pollination mechanisms. In Douglas-fir, no pollination drop is formed and pollen engulfment is involved in pollen transfer while in pine, pollen is transferred from the micropylar arms to the nucellus by pollination drops. A pollination drop is usually exuded from the nucellus in the morning and recedes by midday and not all the ovules in a seed cone exude pollination drops at the same time (Owens and Blake 1984). Doyle and O'Leary (1935) and McWilliam (1958) also reported that pollination drops did not reemerge in a pollinated ovule.

Spruces have a pollination mechanism similar to pines. However, no report is available about the applicability of the first-come, first-served hypothesis. The use of different coloured pollen for each pollination could verify the hypothesis. The present study was designed to determine the importance of the first and second pollinations upon filled seed and the uptake

[1]Revised manuscript received June 20, 1985.

86-03198/

of coloured pollen in white spruce (*Picea glauca* (Moench) Voss).

Materials and methods

Experiment 1

A portion of the polymix pollen (equal volume of clones 432, 446, and 575) was killed in an oven at 100°C for 3 h. Viability of both dead and fresh polymix pollen was tested using the germination technique (Johnson 1943) before controlled pollination.

Seed cones from each of three white spruce clones (S285, S290, and S293) were brush pollinated once or twice when the seed cones reached optimal receptivity (Ho 1984). One pollination or the first pollination was carried out on May 5, 1981, and the second pollination on May 7, 1981. Four combinations were carried out as follows: (*i*) only one pollination with fresh pollen on May 5; (*ii*) two pollinations with fresh pollen; (*iii*) the first pollination with fresh pollen and the second pollination with dead pollen; (*iv*) the first pollination with dead pollen and the second pollination with fresh pollen. Each treatment included 6–10 isolation bags and had at least 20 cones per clone.

Experiment 2

Three colours of pollen were prepared by staining the pollen overnight with food colouring (Food Colour Preparation by Club House Foods Ltd., London, Ont.). The colours were red, green, and blue and the wet pollen was air dried and sieved with very fine mesh screen (opening, 0.175 mm). Some fragmentation of pollen resulted from staining and screening. The pollen viability was not critical because only the relative proportion of coloured pollen entering the micropyle was used to determine the frequency of pollination drop exudation.

Four or more seed-cone buds were isolated before bud burst. The seed cones were then thinned to four of the same developmental stage in at least two isolation bags made for each of four white spruce clones (S283, S285, S291, and S293). Two brush loads of red pollen were applied to each receptive seed cone (May 3, 1981). On the 2nd day, half of the seed cones pollinated with red pollen were brushed with an equal volume of green pollen and on the 3rd day, the other half of the seed cones pollinated with red pollen were pollinated with an equal volume of blue pollen. All the seed cones were collected when they were about to become pendant (May 20, 1981). Cone scales were dissected from the proximal to the distal end of a cone and each of the two ovules on a scale was bisected under a dissecting microscope. The number of each colour of pollen in the micropyle was counted.

Data analysis

The analysis of variance was done following the procedures outlined by Steel and Torrie (1960) and Scheffe's test was used for the multiple comparisons.

Results

The average percentage of filled seeds per cone ranged from 15.3 to 46.7%, depending on the combinations of pollinations (Table 1). Based on Scheffe's multiple comparison, one pollination with live pollen was as effective as two pollinations with live pollen or one pollination with live pollen followed after 1 day with a second pollination with live pollen. However, pollination with dead pollen followed 1 day later by pollination with live pollen gave a significantly lower percentage of filled seeds. Clones and treatments accounted for 50 and 46% of the variation in filled seed set, respectively. The interaction between clones and treatments was not significant. Pollen viability averaged 91% for the polymix pollen and 0% for the killed pollen.

The average number (0.5) of red pollen per ovule was equal for both combinations of pollinations: red pollen followed by either green or blue pollen (Table 2). The average of 0.6 pollen per ovule was obtained for each of green and blue pollen. In a paired *t*-test, there was no significant difference in the treatments between red and green pollen and between red and blue pollen. The analysis of variance indicated that over 60% of the variation accounted for occurred within ramets.

TABLE 1. Average percentage of filled seeds per cone (±SE) from one pollination with live pollen (L), two pollinations with live pollen (LL), first pollination with live pollen and second with dead pollen (LD), and first pollination with dead pollen and second with live pollen (DL) in white spruce

Treatment	Clone			Average
	S285	S290	S293	
L	57.7±4.3*a*	35.8±3.7*a*	45.0±3.8*a*	46.7±2.6*a*
LL	48.6±5.6*a*	39.3±8.2*a*	36.7±4.6*a*	39.5±3.4*a*
LD	56.1±5.7*a*	25.0±4.3*a*	36.8±6.2*a*	39.0±4.1*a*
DL	50.5±4.4*a*	8.5±1.6*b*	6.1±1.3*b*	15.3±2.5*b*

NOTE: Means followed by the same letter do not differ significantly within clone at the 0.5 level.

Discussion

Using brush to transfer pollen onto seed cones, the first pollination resulted in a higher seed set than subsequent pollinations (Table 1). The second pollination with either live or dead pollen had very little effect on the production of filled seeds. The low seed set from the cones pollinated with dead pollen first followed by live pollen could have resulted from the occupancy of dead pollen on most micropylar arms, the unpollinated ovules pollinated in the second pollination, or the reemergence of pollination drops from some of the pollinated ovules. These results agreed with the conclusion in slash pine by Franklin (1974), that the first pollen which reached the cones was most likely to accomplish fertilization. Therefore, one good pollination may be sufficient for a seed cone at its peak of receptivity and contamination as a result of the opening of bags for pollination may not be a major problem because of the first-come, first-served effect.

The brush pollination using coloured pollen resulted in low percentages of ovules with pollen per cone and small numbers of pollen grains per ovule, although many coloured pollen grains were found on the cone scales. This may result from the fragmentation of coloured pollen. An improvement in staining technique should bring good results.

Seed cones which were pollinated with red pollen on the 1st day followed by either green pollen on the 2nd day or blue pollen on the 3rd day had about the same percentage of ovules per cone containing each colour of pollen and the same average number of pollen grains per ovule. This indicated that in a seed cone, ovules without pollen on the nucellus continued to exude pollination drops which picked up the pollen grains from the micropylar arms (Doyle and O'Leary 1935; McWilliam 1958). However, 6.9% out of the 36.5% pollinated ovules contained both red and green pollen and 5.8% out of the 35.4% pollinated ovules contained both red and blue pollen. The appearance of two colours of pollen in an ovule could substantiate the suggestion that the unoccupied site on the micropylar arms was occupied by the pollen from the second pollination and pollination drops were not exuded until after the second pollination. Eventually, pollen of both colours was taken onto the nucellus at the same time.

According to Owens and Blake (1984), formation of pollination drops in Sitka spruce (*Picea sitchensis*) was 6–8 days after the seed cones had fully emerged from the bud scales.

TABLE 2. Average number of coloured pollen grains per ovule and percentage of ovules per cone with coloured pollen grain (±SD) in white spruce

Clone	No. of cones	Total no. of ovules	Average no. of coloured pollen grains per ovule			% of ovules per cone with pollen grains of:			
			Red	Green	Blue	red	green	blue	two colours
				Red pollen − green pollen[a]					
S283	10	1456	0.5±0.2	0.7±0.2		18.9±6.3	24.3±9.0		6.4±3.7
S285	12	1762	0.4±0.2	0.5±0.2		19.4±10.5	23.9±8.5		6.2±3.7
S293	10	1278	0.7±0.4	0.7±0.3		23.4±11.7	20.1±5.9		8.3±3.9
Average			0.5±0.3	0.6±0.3		20.5±9.7	22.9±7.9		6.9±3.8
				Red pollen − blue pollen[b]					
S283	2	300	0.3±0.0		0.9±0.4	15.6±0.8		29.3±9.5	6.1±0.8
S285	12	1872	0.4±0.4		0.6±0.3	19.9±11.5		24.3±7.2	6.3±2.3
S291	6	786	0.4±0.1		0.6±0.2	17.3±4.5		16.6±5.0	4.5±2.4
S293	10	1236	0.5±0.4		0.6±0.3	24.0±11.0		17.2±7.9	5.9±2.6
Average			0.5±0.4		0.6±0.3	20.5±10.0		20.7±8.0	5.8±2.4

[a]Pollination with red-coloured pollen grains followed by green-coloured pollen on the 2nd day.
[b]Pollination with red-coloured pollen grains followed by blue-coloured pollen on the 3rd day.

Using the coloured pollen for pollination in Sitka spruce, they found that some pollen reaching the micropylar arms before exudation was caught and much of it was eventually taken into the ovules. Doyle and O'Leary (1935) and McWilliam (1958) also reported that the presence of pollen on the nucellus induced complete and permanent withdrawal of the drops in pines. However, Lill and Sweet (1977) concluded that in radiata pine (*Pinus radiata*), pollen continued to be transported into micropyles which already contained pollen grains. Somerville and Sweet (1978) also found that most ovules of radiata pine were able to take up pollen of all three applied colours when only one kind of the coloured pollen grains was used to pollinate seed cones on each application at 1- to 4-day intervals of a 6- to 8-day period. The last two studies drew a different conclusion from the others. This may be attributed to the difference in species, the approaches to study pollination mechanism by pollen analysis in the ovules, or repeated pollinations.

Acknowledgement

Appreciation is extended to Dr. J. N. Owens, Department of Biology, University of Victoria, Victoria, B.C., for reviewing the paper and Ms. Marsha Phelps for technical assistance.

DOYLE, J., and M. O'LEARY. 1935. Pollination of *Pinus*. Sci. Proc. R. Dublin Soc. **21**: 181−190.

FRANKLIN, E. C. 1974. Pollination in slash pine: first come first served. *In* Proceedings of a Colloquium: Seed Yield from Southern Pine Seed Orchards. *Edited by* J. Kraus. Georgia Forest Research Council, Macon, GA. pp. 15−20.

HO, R. H. 1984. Seed cone receptivity and seed production potential in white spruce. For. Ecol. Manage. **9**: 161−171.

JOHNSON, L. P. V. 1943. The storage and artificial germination of forest tree pollens. Can. J. Res. Sect. C, **21**: 332−342.

LILL, B. S., and G. B. SWEET. 1977. Pollination in *Pinus radiata*. N.Z. J. For. Sci. **7**: 21−34.

MCWILLIAM, J. R. 1958. The role of the micropyle in the pollination of *Pinus*. Bot. Gaz. (Chicago), **120**: 109−117.

OWENS, J. N., and M. D. BLAKE. 1984. The pollination mechanism of Sitka spruce (*Picea sitchensis*). Can. J. Bot. **62**: 1136−1148.

OWENS, J. N., and S. J. SIMPSON. 1982. Further observations on the pollination mechanism and seed production of Douglas-fir. Can. J. For. Res. **12**: 431−434.

SOMERVILLE, J. G., and G. B. SWEET. 1978. Artificial pollination of *Pinus radiata*. N.Z. For. Res. Inst., Genet. Tree Improv., Intern. Rep. No. 148.

STEEL, R. G. D., and J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, Inc., New York.